A1 contd

22. Furthermore, it is striking that the C-terminal amino acid sequence of the novel inulosucrase contains a putative cell wall anchor amino acid signal LPXTG (SEQ ID No. 5) and a 20-fold repeat of the motif PXX (residues 690-749 of SEQ ID NO: 1) (see figure 1), where P is proline and X is any other amino acid. In 15 out of 20 repeats, however, the motif is PXT. This motif has so far not been reported in proteins of prokaryotic and eukaryotic origin.--

Replace the paragraph beginning at page 5, line 18, with the following rewritten paragraph:

A2

--It was furthermore found according to the invention that certain lactobacilli, in particular *Lactobacillus reuteri*, possess another fructosyltransferase, a levansucrase (FTFB), in addition to the inulosucrase described above. The N-terminal amino acid sequence of the fructosyltransferase purified from *Lactobacillus reuteri* supernatant was found to be QVESNNYNGVAEVNTERQANGQI (residues 2-24 of SEQ ID No. 6). Furthermore, three internal sequences were identified, namely (M)(A)HLDVWDSWPVQDP(V) (SEQ ID No. 7), NAGSIFGT(K) (SEQ ID No. 8), V(E)(E)VYSPKVSTLMASDEVE (SEQ ID No. 9). The N-terminal amino acid sequence could not be identified in the deduced inulosucrase sequence. Also the amino acid sequences of the three internal peptide fragments of the purified fructosyltransferase were not present in the putative inulosucrase sequence. Evidently, the inulosucrase gene does not encode the purified

A2 cont'd

fructosyltransferase synthesizing the levan. The complete amino acid sequence of the levansucrase is shown in SEQ ID No. 11 and the nucleotide sequence is shown in SEQ ID No. 10. The levansucrase comprises a putative membrane anchor (see amino acids 761-765 in SEQ ID No. 11) and a putative membrane spanning domain (see amino acids 766-787 in SEQ ID No. 11). The fructan produced by the levansucrase was identified in the *Lactobacillus reuteri* culture supernatant as a linear (2→6)-β-D-fructofuranan with a molecular weight of 150 kDa. The purified enzyme also produces this fructan.--

Replace the paragraph beginning at page 11, line 8, with the following rewritten paragraph:

A3

--At this time, the N-terminal amino acid sequence of a fructosyltransferase enzyme (FTFB) purified from the *Lactobacillus reuteri* strain 121 was obtained. This sequence consisted of the following 23 amino acids: QVESNNYNGVAEVNTERQANGQI (residues 2-24 of SEQ ID No. 6). The degenerated primer 19ftf (YNGVAEV) (residues 8-14 of SEQ ID NO: 6) was designed on the basis of a part of this N-terminal peptide sequence and primer 20ftfi was designed on the 290 bp PCR product. PCR with primers 19ftf and 20ftfi gave a 754 bp PCR product (see figure 5E), which was cloned into pCR2.1 and sequenced. Both DNA strands of the entire fructosyltransferase gene were double sequenced. In this way the sequence of a 2.6 kb region of the *Lactobacillus reuteri* DNA, containing the

A3 cont'd

inulosucrase gene and its surroundings were obtained.--

Replace the paragraph beginning at page 17, line 1, with the following rewritten paragraph:

A4

--The N-terminal sequence of the purified FTFB was determined and found to be: (A) Q V E S N N Y N G V A E V N T E R Q A N G Q I (G) (V) (D) (SEQ ID No. 6). Three internal peptide sequences of the purified FTFB were determined: (M) (A) H L D V W D S W P V Q D P (V) (SEQ ID No. 7); N A G S I F G T (K) (SEQ ID No. 8); and V (E) (E) V Y S P K V S T L M A S D E V E (SEQ ID No. 9).--

Replace the paragraph beginning at page 20, line 17, with the following rewritten paragraph:

A5

--Figure 1: The nucleic acid (SEQ ID NO: 4) and deduced amino acid sequences (SEQ ID NOS 27 and 1) of the novel inulosucrase of *Lactobacillus reuteri*. Also encompassed within the figure is the comparison peptide (SEQ ID NO: 28). Furthermore, the designations and orientation (< for 3' to 5' and > for 5' to 3') of the primers and the restriction enzymes used for (inverse) PCR, are shown at the right hand side. Putative start codons (ATG, at positions 41 and 68) and stop codon (TAA, at position 2435) are shown in bold. The positions of the primers used for PCR are shown in bold/underlined. The *Nhe*I restriction sites (at positions 1154 and 2592) used for inverse PCR are underlined. The primers used and their exact positions in the inulosucrase sequence are shown in table 1. Starting at amino

A5 cont'd

acid 690, the 20 PXX (residues 690-749 of SEQ ID NO: 1) repeats are underlined. At amino acid 755 the LPXTG (SEQ ID NO: 5) motif is underlined.--

Replace the paragraph beginning at page 21, line 13, with the following rewritten paragraph:

A6

--Figure 3: The N-terminal (SEQ ID NO: 6) and three internal amino acid sequences (SEQ ID NOS 7-9) of the novel levansucrase of *Lactobacillus reuteri*.--

Replace the paragraph beginning at page 21, line 15, with the following rewritten paragraph:

A7

--Figure 4: Parts of an alignment of the deduced amino acid sequences of some bacterial fructosyltransferase genes (SEQ ID NOS 29-40). Sequences in bold indicate the consensus sequences used to construct the degenerated primers 5ftf, 6ftfi and 12 ftfi. (*) indicates a position with a fully conserved amino acid residue. (:) indicates a position with a fully conserved 'strong' group: STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY, FYW. (.) indicates a position with a fully conserved 'weaker' group: CSA, ATV, SAG, STNK, STPA, SGND, SNDEQK, NDEQHK, NEQHRK, FVLIM, HFY. Goups are according to the Pam250 residue weight matrix described by Altschul *et al.* (1990) J. Mol. Biol. 215, 403-410.--